

Genetic Trends Estimation in IRRIs Rice Drought Breeding Program and Identification of High Yielding Drought-Tolerant Lines

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1 **Genetic Trends Estimation in IRRIs Rice Drought Breeding Program and Identification of**
2 **High Yielding Drought-tolerant Lines**

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16

17 **Abstract**

18 **Background**

19 Estimation of genetic trends using historical data is an important parameter to check the success
20 of the breeding programs. The estimated genetic trends can act as a guideline to target the
21 appropriate breeding strategies and optimize the breeding program for improved genetic gains. In
22 this study, 17 years of historical data from IRRI's rice drought breeding program was used to
23 estimate the genetic trends and assess the success of the breeding program. We also identified
24 top-performing lines based on grain yield breeding values as an elite panel for implementing
25 future population improvement-based breeding schemes.

26 **Results**

27 A two-stage approach of pedigree-based mixed model analysis was used to analyze the data and
28 extract the breeding values and estimate the genetic trends for grain yield under non-stress,
29 drought, and in combined data of non-stress and drought. Lower grain yield values were
30 observed in all the drought trials. Heritability for grain yield estimates ranged between 0.20-0.94
31 under the drought trials, and 0.43-0.83 under non-stress trials. Under non-stress conditions the
32 genetic gain of 0.44% (21.20 kg/ha/year) for genotypes and 0.17 % (7.90 kg/ha/year) for checks
33 was observed. The genetic trend under the drought conditions exhibited a positive trend with the
34 genetic gain of 0.11% (1.98kg/ha/year) for genotypes and 0.55% (9.52kg/ha/year) for checks.
35 For combined analysis showed a genetic gain of 0.39% (12.13 kg/ha/year) for genotypes and
36 0.60% (13.69 kg/ha/year) for checks was observed. For elite panel selection, 200 promising lines
37 were selected based on higher breeding values for grain yield and prediction accuracy of >0.40.
38 The breeding values of the 200 genotypes formulating the core panel ranged between 2366.17
39 and 4622.59 (kg/ha).

40 **Conclusions**

41 A positive genetic rate was observed under all the three conditions; however, the rate of increase
42 was lower than the required rate of 1.5% genetic gain. We propose a recurrent selection breeding
43 strategy within the elite population with the integration of modern tools and technologies to
44 boost the genetic gains in IRRI's drought breeding program. The elite breeding panel identified
45 in this study forms an easily available and highly enriched genetic resource for future recurrent
46 selection programs to boost the genetic gains.

47 **Key words: Rice, Drought breeding, Historical data, Genetic trends, Breeding panel**

48 **Background**

49 Rice (*Oryza sativa L.*) is one of the world's major staple crops providing up 20% of the world's
50 dietary energy and feeding more than 3.5 billion people in the world (Wing et al. 2018).
51 Globally, rice is cultivated in an area of 162Mha with an annual production of 755 mt (FAO
52 2019). Amongst the area under rice cultivation, more than 30% is under rainfed ecosystems that
53 are subject to severe drought or water-limited conditions (Dixit et al. 2014). Drought is the major
54 limitation for rice production in rainfed ecosystems leading to yield loss of 13-35% every year
55 (Muthu et al. 2020) and affecting 46Mha of rainfed lowland and 10Mha of upland rice
56 ecosystems in the Asian-Pacific region (Pandey et al. 2007). In Sub-Saharan Africa, drought
57 covers 19% of the total cultivated rice area and is one of the major causes of low rice grain yields
58 (Van Oort and Zwart, 2018). Low grain yield under drought conditions is further elevated by the
59 pressing climatic changes due to the increasing frequency of drought severity events, thus further
60 limiting the rice productivity (Lenaerts et al. 2019).

61 Direct selection for grain yield under drought has been a major focus of the Rainfed Rice
62 Breeding (RRB) program at the International Rice Research Institute (IRRI). Direct selection for
63 grain yield over secondary traits under drought has been proven effective in improving drought
64 tolerance, and as a result, many drought-tolerant rice varieties have been developed (Kumar et al.
65 2014; Sandhu and Kumar, 2017; Kumar et al. 2018; Bhandari et al. 2020; Dar et al. 2020).
66 However, due to the complex nature of grain yield under drought; mainly characterized by the
67 small and large effect genes; their epistatic interactions, and interaction with environment; and
68 other abiotic stresses, genetic improvement in drought has been a major challenge.

69 Despite these challenges, IRRI has been constantly striving to innovate and develop drought-
70 tolerant rice varieties and disseminate them to farmers in Africa and Asia-Pacific regions.
71 STRASA (Stress Tolerant Rice for Africa and South Asia) Project (2005-2019) at IRRI, funded
72 by the Bill and Melinda Gates Foundation (BMGF) was one of the most successful research
73 programs that led to the successful development and release of more than 30 high-yielding
74 drought-tolerant rice varieties in Asia and Africa (<https://strasa.irri.org/>). Under this project,
75 imperative efforts were made to incorporate the major drought-tolerant QTLs (*qDTY1.1*,
76 *qDTY2.1*, *qDTY2.2*, *qDTY3.1*, *qDTY4.1*, *qDTY12.1* *qDTY6.3*, etc.,) into the background of the
77 mega rice varieties like IR64, Swarna, and TDK-1 which led to the development of several high

78 yielding drought-tolerant rice varieties (Bernier et al. 2007; Venuprasad et al. 2009; Vikram et al.
79 2011; Mishra et al. 2013; Yadaw et al. 2013; Sandhu et al. 2014; Kumar et al. 2014; Henry et al.
80 2015; Dixit et al. 2017; Sandhu and Kumar 2017; Henry et al. 2019; Sandhu et al. 2019;
81 Bhandari et al. 2020; Dixit et al. 2020; Kumar et al. 2020; Majumder et al. 2021; Sandhu et al.
82 2021; Yadav et al. 2021). The most popular drought-tolerant rice varieties include - DRR dhan
83 42, CR Dhan 801, Sahbhagi dhan in India, Sukha dhan 4, Bahuguni dhan 11 in Nepal, and
84 Katihan 2, Katihan 3, Sahod Ulan 15, Sahod Ulan 19 in the Philippines, Yaenelo 4 in Myanmar,
85 MPTSA and ATETE in Malawi, CAR 14 in Cambodia, and BRRI Dhan66, BRRI Dhan 71 in
86 Bangladesh (<https://strasa.irri.org/variatal-releases/drought>). Despite these endeavors, and the
87 success of the phenotypic selection coupled with marker-based selection strategies, the progress
88 in the genetic improvement of the drought breeding program has been limited. For example, the
89 average estimated rate of genetic gain in rice drought breeding programs in IRRI-India ranges
90 from 0.68% (under non-stress conditions) to 1.8% (severe drought conditions) (Kumar et al.
91 2021), which is not sufficient to meet future rice demands. Hence, it is crucial to increase rice
92 productivity at a greater rate to ensure food security and prevent potential food crises in the
93 future (Peng et al. 2004; Li et al. 2018). Aiming 1.5% or above genetic gain in rice under drought
94 is a huge challenge and is largely hampered by complex genetics of drought-elevated by extreme
95 climatic changes and increase in the frequency of drought events, and availability of limited land
96 to grow rice.

97 To suffice the increasing food demands, it is important to breed drought-tolerant rice varieties
98 with expected genetic gains. Rice breeders must be smart to implement the advanced tools and
99 technologies into the existing breeding pipeline and re-design it for accelerating genetic gains.
100 The Accelerated Genetic Gain in Rice Alliance (AGGRi) project funded by BMGF is one of the
101 IRRI's ongoing projects aimed at modernizing the IRRI-NARES (National Agriculture Research
102 Extension System) rice breeding program and accelerate the genetic gain from the current level
103 of less than 1% to at least 1.5% or above annually.

104 Genetic gain is an important parameter to check the progress and success of the breeding
105 programs. The genetic gain estimations can be associated with the ongoing breeding program to
106 target the appropriate breeding strategies and act as a guide to optimize and modernize the rice
107 breeding program for accelerated genetic gains. The rate of genetic gain in the IRRI's rice
108 drought breeding program has never been estimated. On the other note, the historical or current

109 elite breeding lines are an important genetic resource that can be directly used in the population
110 improvement-based breeding programs to improve the genetic gains. Further, integrating the
111 modern genomic tools and technologies with the population improvement-based breeding
112 programs using elite lines as a genetic resource will boost the genetic gains (Xu et al. 2017).
113 However, it is important to select the appropriate lines from the historical breeding pool
114 representing the overall genetic diversity in the breeding pool and should have high mean
115 performance for grain yield and possess the major genes or haplotypes for mendelian traits.

116 Thus, to assess genetic gains in the IRRI's rice drought breeding program and select the valuable
117 elite lines as a future genetic resource we conducted this study to 1) estimate the genetic trends
118 for grain yield in IRRI's rice drought breeding program by leveraging 17 years of historical data
119 from the advanced yield trials (AYT) managed under drought and normal conditions and, 2)
120 identify high yielding drought-tolerant lines based on the grain yield breeding values as a future
121 genetic resource for recurrent selection program. The AYT trials included premium released
122 varieties from IRRI globally, therefore, are the potential reservoirs to select some of the high-
123 yielding drought-tolerant lines as a future genetic resource.

124 **Materials and Methods**

125 **Description and Pre-processing of Historical Data**

126 For this study, historical data from yield trials conducted under normal (non-stress) and drought
127 conditions (at the reproductive stage) at IRRI, Philippines from 2003 to 2019 (17 years) were
128 used. The trials in each year were conducted in two seasons- dry (from January to April) and wet
129 (from late June to September) season. The combination of year, season and treatment were
130 treated as a trial or environment. In total the historical data harbored 19,916 data points with
131 2,497 unique lines. The data was pre-processed, and the quality of phenotype records was
132 checked initially to ensure high-quality trials and phenotypes are retained for downstream
133 analysis. The data was checked for extreme or unexpected values, missing percentages, and valid
134 experimental design. The trials having more than 20% of missing data for grain yield, lack of
135 replications, or proper experimental design were dropped initially. Further, the extreme
136 observations were checked in the data before outlier detection as they may increase the error
137 variance which may affect the performance of the outlier detection (González et al. 2018). After
138 preprocessing data was checked for outlier detection using the Bonferroni-Holm test (Bernal-

139 Vasquez et al. 2016; Philipp et al. 2019). The 88 data points detected as outliers were removed
140 from the data to make sure only high-quality data points are retained for reliable estimates. In
141 total 53 trials harboring 19,828 data points with 2,490 unique lines were retained for downstream
142 analysis. The complete information on the trials including the year, season, treatment,
143 experimental design, number of plots, replications, and blocks are provided in the Additional file
144 1: Table S1. Trials were performed in varied experimental designs including alpha lattice,
145 augmented randomized complete block, and randomized complete block designs (RCBD). Three
146 major agronomic traits days to 50% flowering (DTF), plant height (PH), and grain yield (kg/ha)
147 were retrieved and used for downstream analysis.

148 **Pedigree Data Extraction**

149 The pedigree data of 2,490 unique lines were extracted from the breeding data management
150 system (Breeding 4 Results (B4R), 2021) which has the passport to comprehensive information
151 of the genotype, phenotype, and pedigree data of breeding lines. Additional information on the
152 parents and grandparents up to seven generations, cross-type for each line, and breeding strategy
153 were extracted using the IRRI genealogy management system (McLaren et al. 2005; Collard et
154 al. 2019) using customized R scripts.

155 **Statistical Modelling of Phenotypic Data**

156 A two-stage approach of mixed model analysis was used to analyze the data and extract the
157 breeding values for grain yield, DTF, and PH (Piepho et al. 2008; Piepho et al. 2012; Smith and
158 Cullis 2018) under non-stress, drought, and by analyzing drought and non-stress together. The
159 two-stage approach was adopted to account for different experimental designs across the
160 environments (Damesa et al. 2017). In the first stage, per year adjusted means as BLUEs for each
161 genotype were estimated for each environment. The mixed model used consisted of genotypes as
162 fixed effects, and season replications and or blocks were used as random effects. BLUEs for each
163 genotype per year was obtained using the following linear mixed model:

$$y_{ijkl} = \mu + g_i + r_j + b_k + s_l + \varepsilon_{ijkl} \quad (1)$$

164 where, y_{ijkl} represents adjusted means for i th observation in j th replication, k th block and l th
165 season, μ is the overall mean, g_i is the fixed effect of i th genotype, r_j is the random effect of
166 replications in each trial, b_k is the random block effect, s_l is the random effect for season and

167 ε_{ijkl} is the residual error. The random effects were distributed independently and identically. In
 168 this model, DTF was used as a covariate for reducing the error on yield caused due to the
 169 presence of different maturity genotypes. The above model was used for the trials which were
 170 performed using an alpha-lattice breeding design. For environments with augmented RCBD
 171 experimental design, replications were considered equal to blocks, and hence block effect was
 172 removed. Likewise, BLUEs and standard error values were calculated respectively for each
 173 genotype per year for the DTF and PH traits using equation 1.

174 The combined analysis using a linear mixed model was used to extract the single value BLUEs
 175 adjusted across the non-stress and drought treatments. The model used follows as:

$$y_{ijklm} = g_i + r_j + b_k + s_l + t_m + \varepsilon_{ijklm} \quad (2)$$

176 all the terms are described in equation 1 except the t_m which is the fixed effect of m th treatment
 177 (non-stress and drought). We assume different variances across non-stress and drought
 178 treatments in the model to get the adjusted means.

179 Heritabilities for grain yield in each environment across non-stress and drought conditions were
 180 calculated for each environment (Piepho and Mohring 2007). The same model described above
 181 was used to calculate the heritability with genotypes as a random effect. The equation to
 182 calculate heritability follows as:

$$H^2 = 1 - \frac{\bar{V}_{BLUP}}{2\sigma_g^2} \quad (3)$$

183 where, \bar{V}_{BLUP} is the mean-variance difference of two BLUPs and σ_g^2 is the variance of
 184 genotypes.

185 In the second stage analysis, a pedigree-based mixed model approach was used to extract the
 186 breeding values each in non-stress, drought, and combined data using weighted BLUEs as
 187 response variable (Mohring and Piepho 2009). The weighted BLUEs were used to take care of
 188 the heterogeneous error variance and weights were calculated by the inverse of the squared
 189 standard error of BLUEs. The model used was:

$$y_{ij} = \mu + g_i + ye_j + \varepsilon_{ij} \quad (4)$$

190

191 where y_{ij} is the weighted BLUE value for i th observation in j th year, μ is the overall mean, g_i is
192 the random effect of i th genotype with $g_i \sim N(0, A\sigma_g^2)$ where σ_g^2 is the genetic variance and A is
193 the additive genetic relationship matrix based on pedigrees, ye_j is the random effect of year, and
194 ε_{ij} is the residual error, with $\varepsilon_{ij} \sim N(0, R\sigma_\varepsilon^2)$, where R is the identity error covariance matrix and
195 σ_ε^2 is the error variance. The reliability of the breeding values (Isik et al. 2017) of each genotype
196 was estimated using the following equation:

$$r = 1 - \frac{PEV}{\sigma_g^2} \quad (5)$$

197 Two-stage mixed model data analysis was performed in the R software (R Core Team, 2020).
198 using the ASReml-R 4 package (Butler et al. 2017). The R package AGHMatrix was used for
199 constructing the pedigree A-matrix (Amadeu et al. 2016). The analytical pipeline and codes are
200 available on the GitHub (https://github.com/whussain2/Genetic_Trend_Rice_Drought)

201 **Estimation of the Genetic Trends**

202 The genetic gain was estimated separately for three conditions: a) non-stress (normal conditions)
203 trials, b) drought trials, and c) combined data (adjusted means across non-stress and stress trials).
204 For the genetic gain trend, breeding values were regressed on the year of origin of the line. The
205 genetic trend was also estimated for released varieties and checks by regressing the breeding
206 value of checks on the year of origin in non-stress trials, drought trials, and combined data.

207 **Identification of Breeding Panel**

208 Adjusted breeding values obtained from mixed-model analysis across non-stress and stress data
209 were used for the identification of elite genotypes as a future breeding resource. A total of 200
210 lines were selected from the 2,490 unique historical lines based on the higher breeding values
211 and prediction accuracy of >0.4 . In addition to the lines with higher breeding values and
212 prediction accuracy for grain yield, lines with key QTLs for key biotic and abiotic stresses. To
213 make sure genotypes selected are diverse and represent the whole collection of lines in historical
214 lines, the pedigree matrix was used in the analysis to account for similarity among the lines. The
215 similarity and diversity among the selected lines in comparison to the whole collection were
216 visualized through bi-plot. For bi-plot, principal component analysis (PCA) was performed on
217 the pedigree-based relationship matrix using the *princomp()* function in R software. Bi-plot was
218 visualized using the *factoextra* R package (Kassambara and Mundt, 2017)

219 **Results**

220 **Descriptive Features of Historical Drought Data**

221 The three main traits grain yield, PH, and DTF grown under normal and drought conditions were
222 used for analysis. The difference in phenotypic trait values for all three traits was observed in
223 normal and drought conditions. The difference in trait value was also evident across the years.
224 The raw mean grain yield under the normal conditions ranged from 3000-12000 (kg/ha) as
225 compared to the drought conditions 1360-5600 (kg/ha) (Fig. 1a). In each trial, lower yield values
226 were observed under drought conditions as compared to trials under normal conditions indicating
227 the impact of drought on the phenotypes. For DTF we observed higher values under drought
228 conditions (63-129 days) as compared to the trials under normal conditions (60-119 days)
229 (Kazan and Lyons 2016; Shavrukov et al. 2017). Further, we observed a wide distribution in
230 DTF for the genotypes, and genotypes were classified into three maturity groups i.e., early (85-
231 109 days), medium (110-124 days), and late (DTF>125 days) (Additional file 1: Fig. S1). The
232 DTF of 68% of the lines from the complete unique set of lines under drought implication falls
233 into the medium duration maturity category. The remaining genotypes make up 15% and 16%
234 for early and late duration groups, respectively. Similarly, under the non-stress conditions, the
235 percentage of lines falling into three maturity categories were, early (15%), medium (76%), and
236 late (9%) respectively. Because of the wide distribution in DTF, we used DTF as covariate in the
237 phenotypic data modeling to adjust for the grain yield. Similarly, for PH we observed a wide
238 distribution in phenotypic values and PH in the data set ranged from 40-195 (cm) under normal
239 conditions and 40-90 (cm) under drought conditions (Additional file 1: Fig. S2). The dataset has
240 ample diversity among the tested genotypes having a diverse range for PH between 40 cm to 195
241 cm. Low plant height was observed under drought conditions, consistent with the previous
242 reports (Ahmadikhah and Marufinia 2016; Mishra and Panda 2017; Hussain et al. 2018; Panda et
243 al. 2021). Heritability for grain yield estimates ranged between 0.20-0.94 under the drought trials
244 and 0.43-0.83 under normal growing trials for the non-stress trials (Fig. 1b). Lower heritability
245 was observed in most of the drought trials. Reduction in heritability under drought conditions is a
246 common phenomenon (Henry et al. 1997, Kumar et al. 2007) which indicates that genotypes are
247 not able to express the higher genetic potential for grain yield.

248 **Historical Data Connectivity**

249 Historical data sets usually have very low connectivity as new lines are being tested every year,
250 and only a limited number of times the new lines are being tested. In the current data set, we
251 observe appropriate connectivity of the different genotypes across the years (Fig. 1c), and this
252 connectivity was mainly created by long-term checks (IR64, Swarna, Sahbhagi Dhan, IRRI 154)
253 across the years. Further, over the breeding cycles and years, the superior genotypes were
254 forwarded and re-tested in the succeeding years which made the dataset well connected to
255 previous years (Additional file 1: Fig. S3) Further to ensure good connectivity and get reliable
256 estimates of the breeding values, relationship matrix (Additional file 1: Fig. S4) based on
257 pedigrees of 2,970 unique lines was incorporated in the second stage of mixed model analysis.

258 **Estimation of Breeding Values**

259 Breeding values obtained from second-stage analysis by fitting a pedigree matrix were used to
260 estimate the genetic trends and used to identify the best lines based on higher breeding values for
261 the formulation of the core panel. The range of the breeding values for the genotypes under
262 drought stress was between 642.79- 3,267.60 (kg/ha). Under the normal growing conditions, the
263 breeding values of the genotypes ranged between 3,447.93- 6,933.32 (kg/ha). The breeding
264 values adjusted across drought and normal growing conditions ranged between 1,026.93-
265 4,622.59 (kg/ha). The histogram of the breeding values along with the mean and standard
266 deviations is given in the (Additional file 1: Fig. S5).

267 **Estimation of Genetic Trends**

268 The genetic trend was estimated for the genotypes and, also for checks and released varieties in
269 normal growing conditions, drought conditions, and in combined data. Under normal growing
270 conditions, the genetic gain of 0.44% with a yield advantage of 21.20 kg/ha per year was
271 observed for genotypes (Fig. 2a), and genetic gain of 0.17% was observed for checks and
272 released lines representing increase of 7.90 kg/ha per year (Fig. 3a). The genetic trend under the
273 drought conditions exhibited a positive trend with a genetic gain of 0.11% for genotypes (Fig.
274 2b) and 0.55% for released lines (Fig. 3b). Yield advantages of 1.98 kg/ha for genotypes and
275 9.52 kg/ha for checks was observed. The regression estimates for combined analysis (adjusted
276 breeding values across normal and drought growing conditions) showed a genetic gain of 0.39%
277 for genotypes (Fig. 2c) and 0.60% (Fig. 3c) for checks with a yield advantage of 12.13 kg/ha for
278 genotypes and 13.69 kg/ha for checks.

279 Further we divide the genetic gain trend estimation in time periods of 2003-2012 and 2013-2019
280 based on the breeding strategies adopted by the drought breeding program at IRRI. Genetic gain
281 was 0.31 % (under non-stress condition) and 0.21 % (under stress condition) higher in the period
282 2013-2019 as compared to period 2003-2012 (Additional file 1: Fig. S6)

283 **Comparison of Breeding Values**

284 We compared the breeding values of the popular checks and the released varieties from IRRI, to
285 assess their performance under three conditions *viz.*, stress, non-stress, and combined stress and
286 non-stress. This assessment was undertaken with the overview to deduce the best performers
287 among the released IRRI lines. The performance of the released varieties was superior to the
288 popular checks under all three conditions (Fig. 3a-c). The varieties IRRI 188, IRRI 199, and
289 IRRI 200 had superior breeding values of 2,547.19 (kg/ha), 2,420.70 (kg/ha), and 2,490.97
290 (kg/ha), respectively, compared to popular checks and other released varieties when estimated
291 under the drought stress conditions (Fig. 3a). These varieties were released in the years 2015 and
292 2016, depicting growth of the breeding program over the preceding released varieties developed
293 in the previous years. The latter also showed the higher performance to the most popular
294 drought-tolerant released variety Sahbhagi Dhan with the predicted value of 1,562.03 (kg/ha)
295 under stress conditions. The breeding values for popular drought-tolerant checks Vandana and
296 Rajashree were 1,391.68 (kg/ha) and 1815.65 (kg/ha) under drought stress conditions
297 respectively. This depicts the superiority of the recently released lines over popular checks and
298 varieties. The genotypes were also assessed for the trials evaluated under non-stress conditions.
299 The breeding lines, IR09L204 depicted the highest breeding value of 4998.44 (kg/ha) followed
300 by IRRI 199 (4,958.74), IRRI 218 (4,900.64), IRRI 200 (4,892.13), and IRRI 188 (4,866.45) in
301 the descending fashion of the superior performing genotypes under the non-stress conditions
302 (Fig. 3b). In the combined analysis with breeding lines performing superior under both drought
303 stress and non-stress conditions were as follows IRRI 119 (3,698.75), IRRI 163 (3,599.56), IRRI
304 162 (3,395.07), IRRI 218 (3,374.91), and IRRI 200 (3374.93) (Fig. 3c). Similar to stress
305 conditions, these lines surpassed popular checks Vandana (2,814.09) and Rajashree (2,628.67);
306 variety Sahbhagi Dhan (2,668.12). The breeding lines of the five top-performing lines have been
307 detailed in Fig. 3a-c. for stress, non-stress, and combined conditions, respectively.

308 **Identification and Development of Elite Breeding Panel**

309 The genotypes with higher breeding values based on the grain yield and with prediction accuracy
310 of >0.40 were selected for the development of the breeding core panel. In total 200 promising
311 lines were identified and used for the development of the elite panel. To make sure 200 selected
312 lines are genetically diverse and representative of decades of IRRI's drought breeding and
313 varietal development, a pedigree matrix was used to account for genetic similarity among the
314 genotypes. The relationship matrix constructed using pedigree data was visualized using the
315 biplot (Fig. 3d). From the biplot, it is very clear how diverse the selected lines are, and how they
316 represent and capture the diversity of the whole historical collection of 2,490 unique genotypes.
317 The breeding values of the 200 genotypes formulating the core panel ranged between 3,200 to
318 4,622.59 kg/ha. The mean breeding value of the panel is 3,395.10 with a standard deviation of
319 3,73.23 (Additional file 1: Fig. S7).

320 **Discussion**

321 Here we provide an overview of how 17 years of historical IRRI's rice drought data was
322 leveraged to estimate the breeding values for grain yield and estimate the genetic trends in the
323 IRRI's rice drought breeding program. We also demonstrated how the top-performing lines
324 based on the grain yield breeding values were selected as the future breeding elite resource for
325 recurrent selection-based breeding. For the selection of genotypes as a part of the core panel,
326 pedigree information availability was pivotal in fitting the additive matrix in the second stage of
327 the mixed model analysis for reliable estimation of the breeding values and help in the selection
328 of accurate genotypes for the formulation of the core panel. The essence of using the relationship
329 matrix is that it contains information about the flow of genes and explicitly allows the dissection
330 of genetic variation by accounting for the additive genetic covariances between random
331 effects/genotypes for reliable estimation of the breeding values (Piepho et al. 2008). Further, the
332 relationship matrix ties up the data across years by borrowing information from parents and
333 grandparents and creating the connectivity in the highly unbalanced data set for the reliable
334 estimation of breeding values.

335 **Genetic Trend and Breeding Value Estimations**

336 Improving the crop yield or genetic gain is crucial for minimizing the "yield gap" between the
337 breeders and farmers. The assessment of genetic gains to estimate crop yield growth has had a
338 limited focus in the past. However, genetic gain estimation with an outlook to reinforce the

339 future breeding programs to increase genetic gain for yield has become a major focus. In this
340 study, the regression of breeding values over the year of release/testing indicated a positive
341 genetic gain under all three conditions, and the overall success of the drought breeding program
342 at IRRI. However, the genetic gain of 0.11% observed under drought conditions is not sufficient
343 to meet the current and future rice food demands. A much higher genetic gain of 1.9% was
344 reported under severe drought conditions at the reproductive stage in rice evaluated in IRRI India
345 (Kumar et al. 2021). Therefore, it is essential to optimize and modernize the drought rice
346 breeding program at IRRI for enhanced genetic gains.

347 Further we observed a minimal increase in genetic gain increase till the year 2012, post which
348 there was higher increase in genetic gain (Additional file 1: Fig.S6) which could be accredited to
349 differential breeding strategies followed in the drought breeding program across the years. The
350 drought breeding program at IRRI until 2012 was led specifically for targeting the introgression
351 of major abiotic stress-tolerant QTLs/genes for the development of NILs possessing elite genetic
352 backgrounds with minimal focus on recurrent selection breeding strategy. Thereafter, in the
353 preceding years, the focus drifted towards pyramiding these genes/QTLs for rendering multiple
354 stress-tolerant cultivars. These genotypes were not precisely targeted for genetic gain
355 enhancement; however, their genetic merit is highly valuable as these sustainable varieties were
356 disseminated for commercial cultivation across countries and can withstand additional biotic and
357 abiotic stresses along with drought. Further in the last few years more emphasize was given to
358 recurrent selection-based breeding strategies in the drought breeding program to improve the
359 yield and increase genetic gain. Rather than the QTL-based introgression or breeding approach
360 as used previously, rice breeders need to focus on population improvement breeding approaches
361 using elite lines as parents, wherein parents of each breeding cycle are selected based on high
362 additive breeding values for the grain yield. Recurrent selection schemes focused on quick
363 recycling of the best and high breeding value lines may deliver higher rates of genetic gain
364 (Cobb et al. 2019). Further to ensure the constant genetic gains, rice breeders need to select the
365 parents for new breeding cycles that have higher additive breeding value than the previous
366 breeding cycle. However, for long-term genetic gains, utmost care must be taken by the breeders
367 to diversify the elite gene pool by bringing or directly crossing the exotic or diverse materials
368 with elite pool lines. Exotic or diverse materials may broaden the elite gene pool, but they are
369 highly unadopted and unimproved lines with lower breeding values. Thus, crossing a directly

370 diverse line with an elite line may bring novel favorable alleles and increase the genetic variance
371 of progeny, however, they barely counterbalance the mean performance of progeny due to the
372 low breeding value of diverse or exotic lines (Longin et al. 2014; Allier et al. 2020). Thus, a
373 focused recurrent selection breeding approach with a systematic pre-breeding approach is
374 required to deliver higher and constant genetic gains in IRRI's drought rice breeding program.

375 The drought breeding program at IRRI has successfully released many drought-tolerant varieties
376 across Asia and Africa (<https://strasa.irri.org/>), and most of them were part of this study. We
377 separately assessed the genetic gain estimation for released varieties, and higher genetic gain was
378 observed for varieties/checks released for use under drought conditions. The positive and higher
379 genetic gain (0.55%) for checks under drought indicates that a strong impact has been made by
380 IRRI's drought breeding program to increase rice productivity under challenging and extreme
381 environments. Among these varieties- IRRI 188, IRRI 199, and IRRI 200 released during 2015
382 and 2016 had higher breeding values as compared to the popular checks Rajashree, Vandana, and
383 UPLRi7, and previously released varieties. The superiority of these lines over the formerly
384 released varieties indicates growth in grain yield to a larger extent. Also, positive genetic trend
385 and higher breeding values of the recently released varieties demarcates the positive growth of
386 the breeding program across years as the performance improved from the preceding released
387 varieties developed in the previous years. Further, among these three top-performing released
388 lines, IRRI 199 originates from a backcross breeding program utilizing a tropical japonica
389 drought and blast tolerant genotype, Moroberekan, as a donor parent and a high-yielding, semi-
390 dwarf Indica rice variety, Swarna. The population harbors a major, severe drought-tolerant QTL,
391 *qDTY3.2* contributing to various drought-tolerant traits *viz.*, canopy cover, canopy temperature
392 (CT), root system architecture (RSA) attributes (Wasaya et al. 2018; Sofi et al. 2019). The
393 genetic region co-localizes with early flowering time QTL *HD9* and lodging resistance features,
394 making the genotype suitable for various ecosystems and environments (Dixit et al. 2014; 2015).
395 Concomitantly, based on the combined analysis IRRI 163, IRRI 162, IRRI 218 and IRRI 200
396 released in the years 2011, 2015, and 2016 exhibited high breeding values under both stress and
397 non-stress conditions.

398 **Development of Breeding Panel as The Future Breeding Resource**

399 In the last one-decade rice breeders at IRRI have mainly focused on introgression and
400 pyramiding of major abiotic stress-tolerant QTLs/genes in elite backgrounds (Venuprasad et al.
401 2009; Mishra et al. 2013; Yadav et al. 2013). Population improvement based on recurrent
402 selection and early re-cycling of advanced lines has not been a major focus of the drought rice
403 breeding program at IRRI. Different crossing strategies (Additional file 1: Fig. S8) single,
404 complex, double, and backcrosses have been used by the rice breeders to integrate these QTLs
405 into the elite genetic backgrounds and develop the new breeding lines. Diverse materials,
406 including landraces, and donors have been extensively used to diversify the gene pool and
407 develop climate-resilient varieties (Sandhu et al. 2021, Yadav et al. 2021). However, this strategy
408 of diversifying the elite gene pool with limited focus on recurrent selection and early re-cycling
409 of high-value breeding lines may have limited genetic gain to a large extent and has not been
410 sufficient to maintain the higher genetic gains over time.

411 Recurrent selection with early re-cycling of lines is the key to increase the frequency of desirable
412 additive haplotypes of grain yield in each cycle, and ultimately boosting the genetic gains. To
413 strictly focus on recurrent selection breeding schemes, the presence of highly characterized elite
414 lines with higher breeding values for grain yield and possessing the key haplotypes for
415 mendelian traits is required as the base population. The historical data set used in this study
416 which contains 2,497 unique genotypes has been used by rice breeders at IRRI for decades and
417 in the past 60 years, many promising drought lines have been extracted from this breeding pool.
418 This breeding pool exhibits ample genetic diversity and possesses the key lines that may be used
419 as a future breeding resource to sustain higher grain yield under challenging environments.
420 Further, this breeding collection has not only been improved for grain yield but also
421 turbocharged with diverse alleles for important traits of biotic and abiotic stresses and represents
422 the overall diversity and breadth of IRRI's rice drought breeding program. Besides the gene bank
423 resources, this breeding pool represents the important source of genetic variation that is highly
424 dynamic created through recombination and reshuffling of alleles. Thus, identifying the high-
425 power performing lines based on the breeding values for grain yield that represents the overall
426 diversity of the whole breeding collections is the key to success in future recurrent selection
427 breeding strategies. To this end, we took this initiative to extract the top breeding lines from the
428 whole historical breeding collection and form the elite breeding panel as future breeding
429 resources. These selected lines besides possessing high breeding values are also indicative of

430 higher recovery capabilities under drought stress. We believe the lines selected are the best
431 genetic variation to recombine and reshuffle in recurrent selection to increase the frequency of
432 additive haplotypes of grain yield in each cycle of breeding. Additionally, based on the literature
433 we assessed the additional characteristic features of these selected lines for resistance to biotic
434 stresses (bacterial blight, blast, brown planthoppers, stemborer, whiteheads, green leafhoppers,
435 and rice tungro virus), drought tolerance, and quality traits (Additional file 1: Table S2). It is
436 evident from the table that these lines harbor the key genes and is a readily available elite genetic
437 resource for future recurrent selection breeding schemes for targeting preferred
438 environments/countries based on the desirable market profiles.

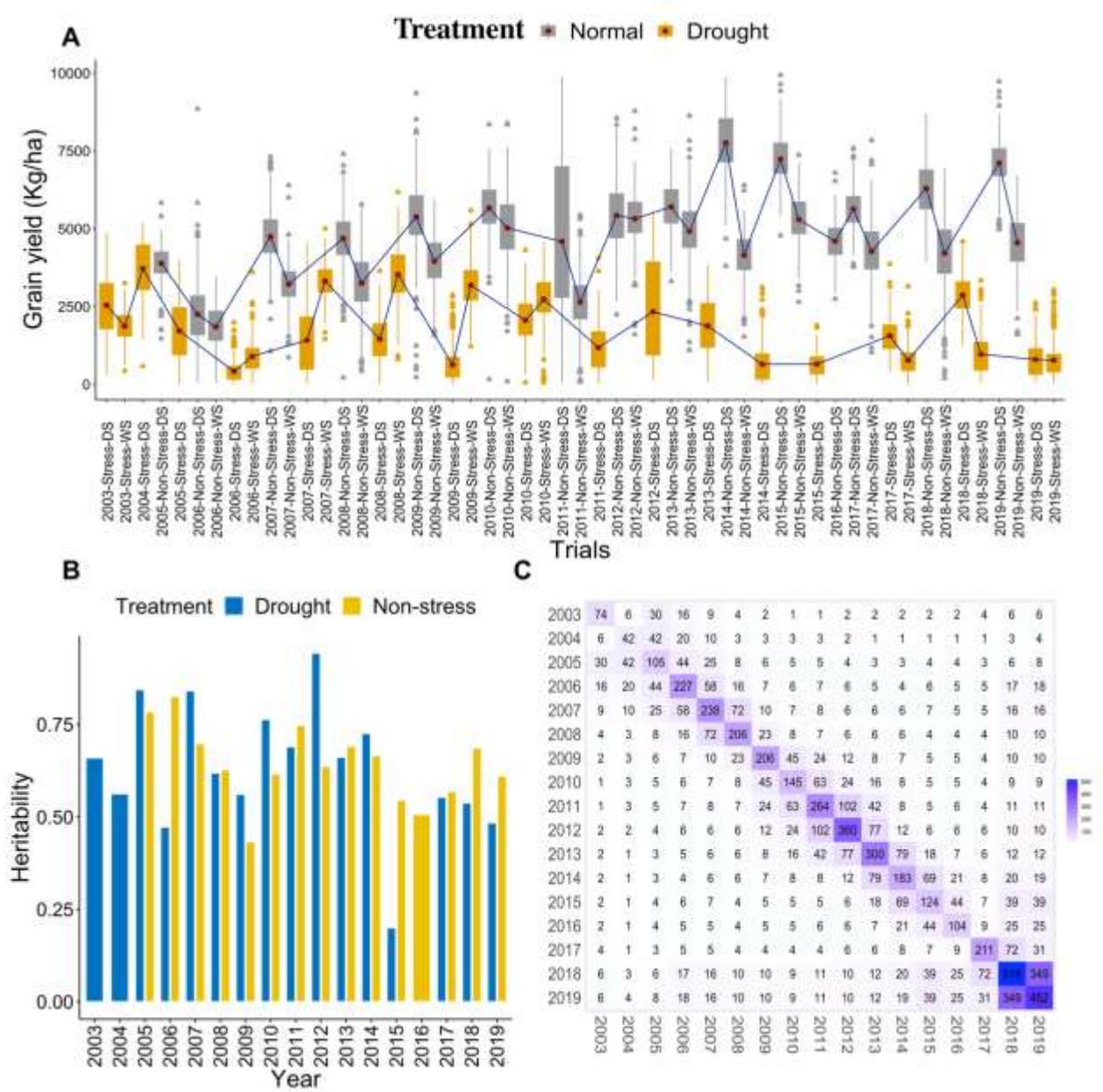
439 Additionally, the elite panel also harbored two best performing genotypes; IR15F1706 and IR
440 54447-3B-10-2 from the 2020 drought stress trials (unpublished) showing high chlorophyll
441 fluorescence (CF) and low CT values. These genotypes when assessed had high breeding values,
442 confirming further the accuracy of the analysis undertaken. Alongside, it also demarcates that
443 deprivation in CT has a strong influence on plant's yield under drought-prone conditions. It has
444 also been reported that CT has a high correlation with the RSA traits symbolizing enhanced
445 genetic capacity of the plant to retain soil moisture and hence improved survival and yield under
446 drought stress conditions (Blair et al. 2010; Lopes and Reynolds 2010). Similarly, enhanced CF
447 demarcates a plant's capabilities to withstand drought stress effectively. Furthermore, two
448 multiparent conventional bred lines namely, IR 115844-B-B-281-1-2 and IR 115844-B-342-1-1-
449 1 present in the selected panel have been reported to yield more than 7000 kg/ha under dry
450 direct-seeded conditions, with higher yields under non-stress and reproductive stage drought
451 stress conditions (Sandhu et al. 2021). In our study, these lines showed the breeding values
452 between 3200 to 3368.14, which form a valuable resource for the breeding programs to be
453 utilized for drought-prone areas with major cultivation under dry seeded conditions having
454 limited water and labor availability. Few of the top 100 selected lines were also reported by other
455 research studies to show higher yield performance under multi-environment trials (Vergara et al.
456 2016).

457 In summary, the core breeding panel selected based on the breeding values and prediction
458 accuracy is an important genetic resource possessing multiple stress tolerance, varied range of
459 quality traits with genotypes suited for cultivation under challenging environments. Furthermore,
460 they form an easily available and highly enriched genetic resource for future recurrent selection

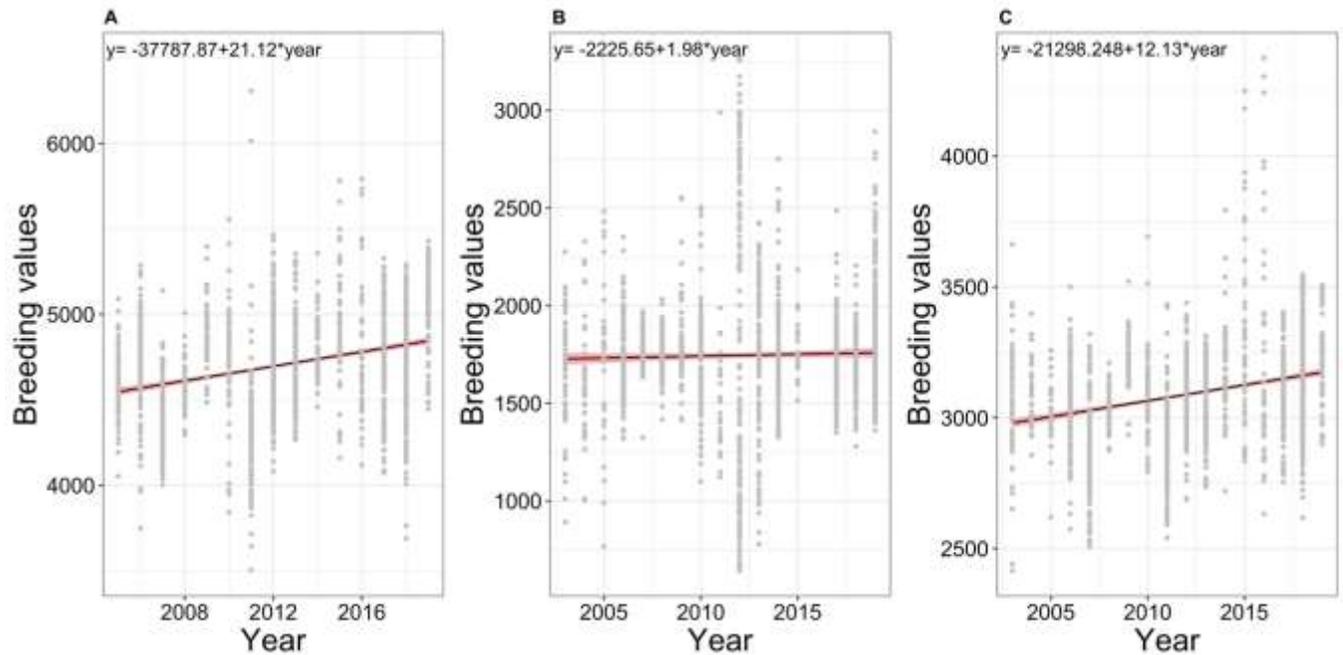
461 programs and enhance genetic gains. However, we emphasize systematic genotypic and
462 phenotypic characterization of these lines in achieving more knowledge on the value proposition
463 of these lines, new allele enrichment, and help to create a framework for better understanding
464 and managing the genetic diversity in the elite pool. However, the question may arise whether
465 continued use of the elite pool lines and reshuffling of alleles in closed recurrent selection
466 strategies is enough to maintain long-term genetic gains? Most will agree with the enrichment of
467 the elite breeding pool with diverse materials as was done previously in the IRRI's drought
468 breeding program. However, we emphasize here a systematic effort to diversify the elite gene
469 pool without contaminating it with diverse materials and limiting the genetic gains (Allier et al.
470 2020).

471 **Conclusion**

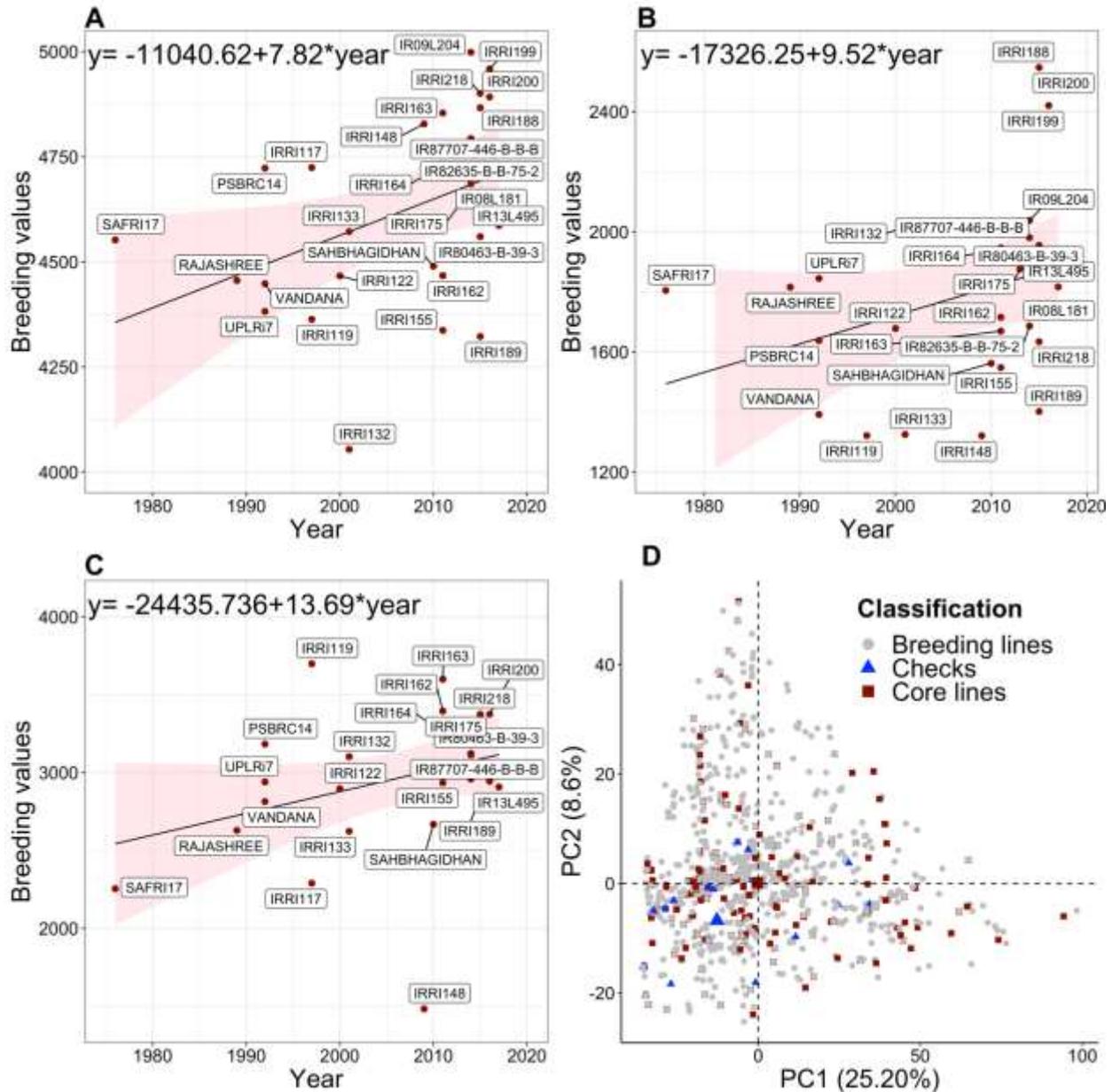
472 The drought breeding program at IRRI has been successful in maintaining a positive genetic rate
473 in the breeding program, however, the increase in genetic gain has not been so high to fulfill the
474 rice food demands. To achieve the required genetic gains of 1.5% or above, a recurrent selection
475 breeding strategy of the elite population with the integration of modern tools and technologies is
476 needed. Genotypic and phenotypic characterization of the selected elite panel is required to
477 effectively manage, incorporate, and track the genetic diversity for short-term and long-term
478 genetic gains. Further, efficient pre-breeding strategies are needed to turbocharge the elite gene
479 pool with major haplotypes of traits showing discrete Mendelian segregation without
480 compromising the performance of elite lines and boost the genetic gains.



482
 483 **Figure 1:** (a) Boxplot showing the mean grain yield (kg/ha) under normal and drought conditions
 484 from the year 2003 -2019. The x-axis shows the trial names, which are combinations of year,
 485 season, and growing condition. In the boxplots, it is clear the grain yield is higher under normal
 486 conditions as compared to drought conditions indicating the impact of drought on the yield trials.
 487 (b). Heritabilities of the trials in each year from 2003 to 2019. The blue bars represent drought and
 488 yellow non-stress trials. (c). Connectivity of all the genotypes across years from 2003 to 2019. The
 489 genotypes including common checks and promising varieties were repeatedly tested for their
 490 performance in the successive years, thereby making the dataset well connected across successive
 491 years. The numbers in the boxes show the genotypes that were common between years.



492
 493 **Figure 2:** Trends in genetic gain from IRRI's 17 years of drought breeding program under. a) Stress
 494 conditions, b) non-stress conditions, and c) combined conditions (adjusted breeding values under stress
 495 and non-stress conditions). The x-axis shows the year of origin of the genotype and the Y-axis shows the
 496 breeding value of the genotype. The genetic gain was estimated by regressing the breeding values of
 497 grain yield on the year of origin and is given by the slope of the line.
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Figure 3: Trends in genetic gain for IRRIs drought released lines and popular checks under three conditions: a) non-stress, b) reproductive drought and c) combined conditions. The genetic gain was estimated by regressing the breeding values on the year of origin, d) shows the biplot of the lines selected based on the breeding value for grain yield as an elite core panel. Core panel lines are highlighted in dark red color. The checks and released lines are shown in blue color, and the whole historical collection lines are represented in gray color. The biplot was constructed using the first two principal components obtained from the pedigree-based relationship matrix. The selected lines represent and capture the variability of the whole collection of genotypes and are ideal to form the core panel as a future breeding resource.

511 **Availability of Data and Materials**

512 The datasets included in this work are given as additional files. The additional data and scripts
513 used to run the analysis can be found on the GitHub page at the following link:
514 <https://github.com/whussain2/Genetic Trend Rice Drought>

515 **Abbreviations**

516 BLUP: Best linear unbiased predictions
517 BLUE: Best linear unbiased estimates
518 REML: Residual maximum likelihood
519 GEBV: Genomic estimated breeding values
520 CV: Coefficient of variation
521 AYT: Advanced yield trials
522 PH: Plant height
523 DTF: Days to 50% flowering
524 PCA: Principal component analysis
525 QTL: Quantitative trait loci
526 MABB: Marker-assisted backcross breeding
527 RSA: Root system architecture
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762 WH and SB designed the study, conceived the concept, and provided overall guidance in
763 conducting the study. AK and WH performed all the statistical analysis. AK wrote the initial
764 draft of the manuscript. MA and MC helped in data collections, analysis and drafting the
765 manuscript. JB, RFN and JDP helped in editing and reviewing the manuscript and provided
766 overall guidance in the data analysis. JB, DJP and AG helped in the data analysis and extraction
767 of pedigrees for data. TSC, JR and GF helped in data collections and drafting the manuscript. All
768 the authors read, reviewed, and approved the final manuscript.

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774 **Ethics declarations**

775 **Ethics approval and consent to participate**

776 Not applicable

777 **Competing interests**

778 The authors declare that they have no competing interests.

779 **Publisher's Note**

780 **Supplementary Information**

781 **Genetic Trends Estimation in IRRIs Rice Drought Breeding Program and Identification of**
782 **High Yielding Drought-tolerant Lines**

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795

796 **Additional file 1: Figure S1:** Boxplots showing distribution of raw data values for days to 50%
797 flowering (DTF) data under non-stress and drought conditions.

798 **Additional file 1: Figure S2:** Boxplots showing the distribution of raw data values for plant
799 height(cm) data under non-stress and drought conditions.

800 **Additional file 1: Figure S3:** Depiction of the number of same genotypes tested across the years
801 in the breeding program.

802 **Additional file 1: Figure S4:** Pedigree-based heat map and clustering of the genotypes bred over
803 the years.

804 **Additional file 1: Figure S5:** Distribution of the breeding values for grain yield.

805 **Additional file 1: Figure S6:** Shows genetic trends from the year 2005- 2012 under non-stress
806 conditions, b) shows genetic trends from the year 2013- 2019 under non-stress conditions, c)
807 shows genetic trends from the year 2003- 2012 under stress conditions, and d) shows genetic

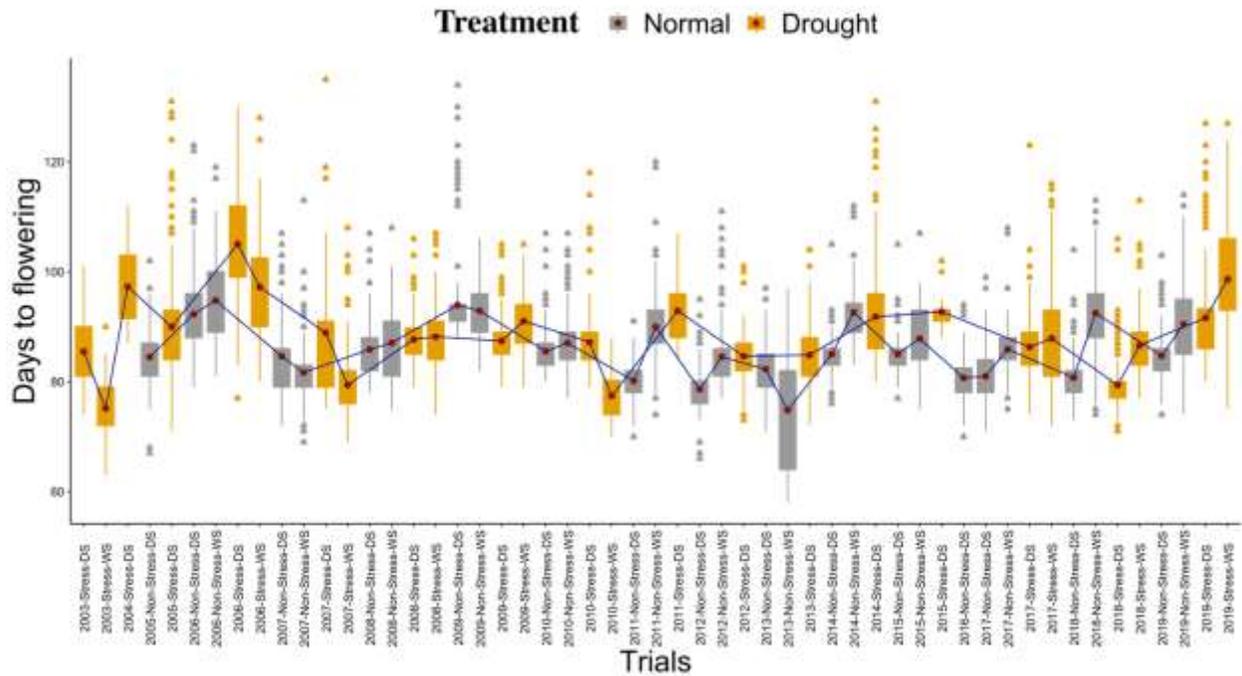
808 trends from the year 2013- 2019 under stress conditions. Both under non-stress and stress
809 conditions higher genetic gain was observed post to year 2012.

810 **Additional file 1: Figure S7:** Distribution of 200 selected genotypes for grain yield breeding
811 values.

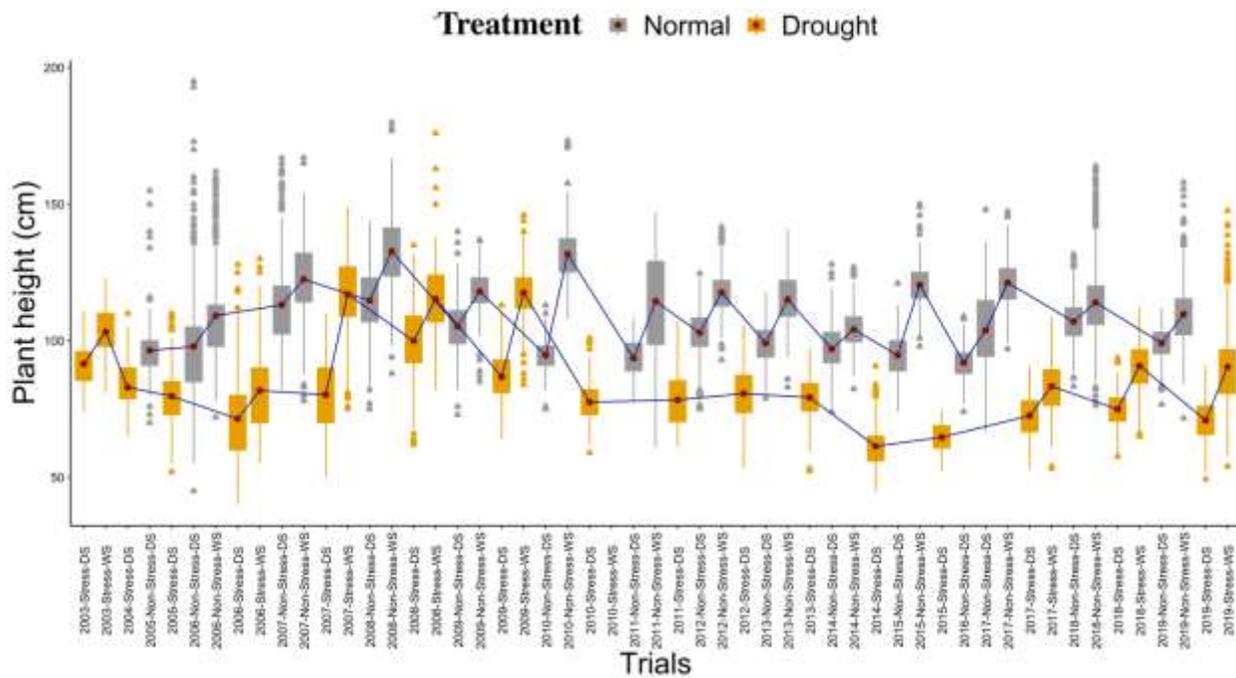
812 **Additional file 1: Figure S8:** Breeding schemes implemented each year in the drought breeding
813 program from the year 2003-2019.

814 **Additional file 1: Table S1:** List of trials used in this study for genetic trend estimations and
815 formulation of elite core panel.

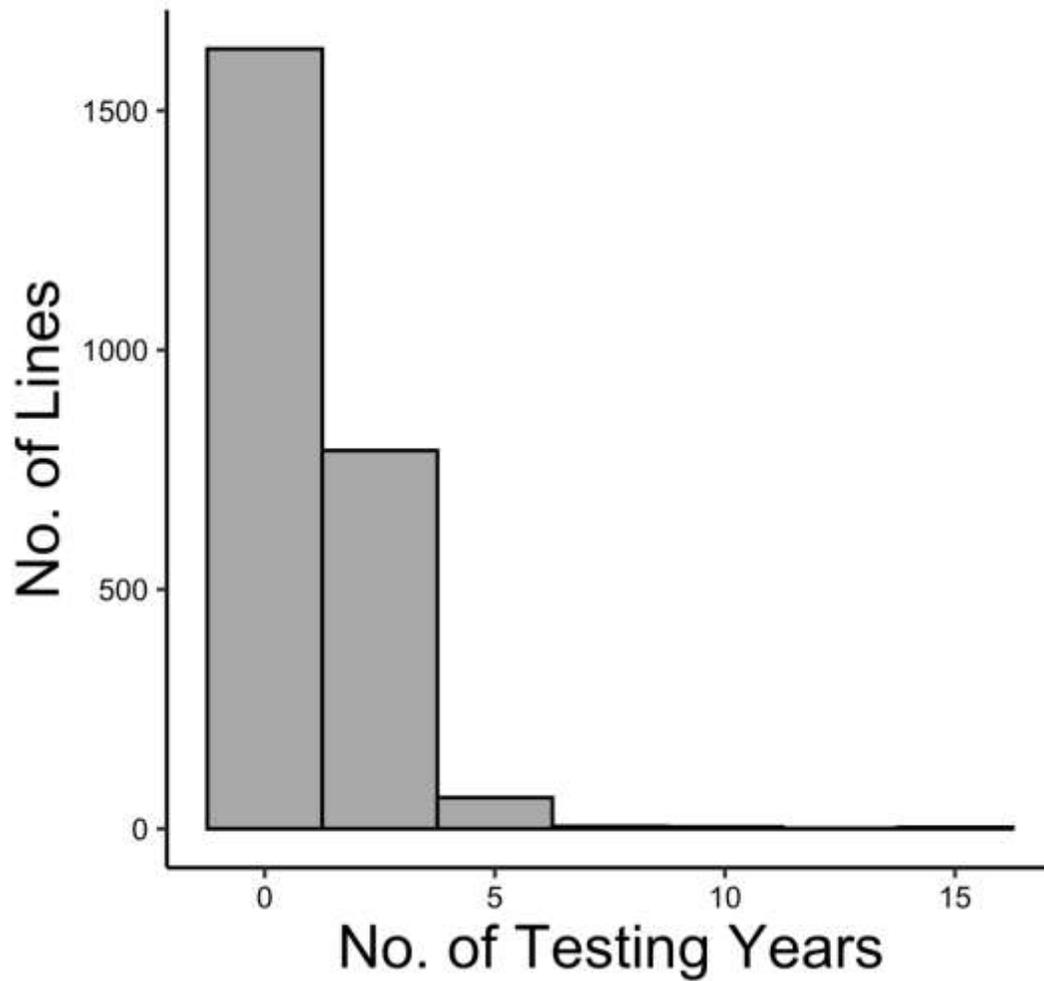
816 **Additional file 1: Table S2:** Delineation of the traits and characteristics of top performing
817 genotypes formulating the breeding panel.



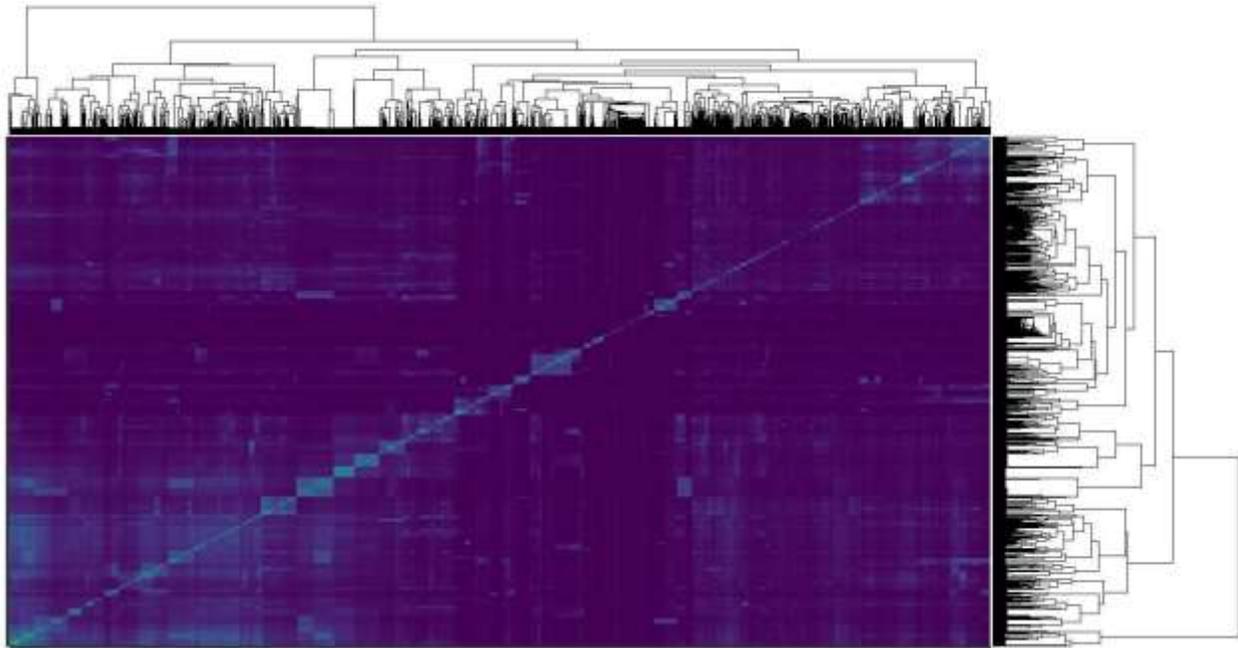
818
 819 **Figure S1:** Boxplots showing the raw days to 50% flowering (DTF) data under non-stress
 820 (normal) conditions and drought conditions from the year 2003-2019. The Plant height values of the
 821 non-stress trials are illustrated in gray, as a comparison to the Plant height values of the stress
 822 trials denoted in orange.



823
 824 **Figure S2:** Boxplots showing the raw plant height (cm) data under non-stress (normal)
 825 conditions and drought conditions from the year 2003-2019. The plant height values of the non-
 826 stress trials are illustrated in gray, as a comparison to the plant height values of the stress trials
 827 denoted in orange.

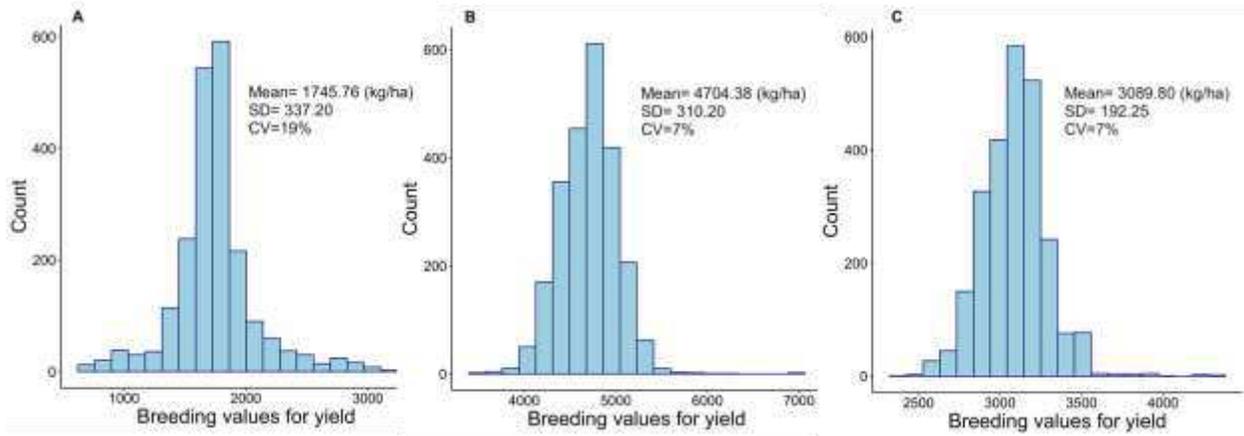


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 829 **Figure S3:** The figure depicts the number of the same genotypes tested across the years. It is
 830 apparent from the figure that a limited number of genotypes were evaluated for more than one
 831 year. Less than 50 genotypes were evaluated for more than two years.



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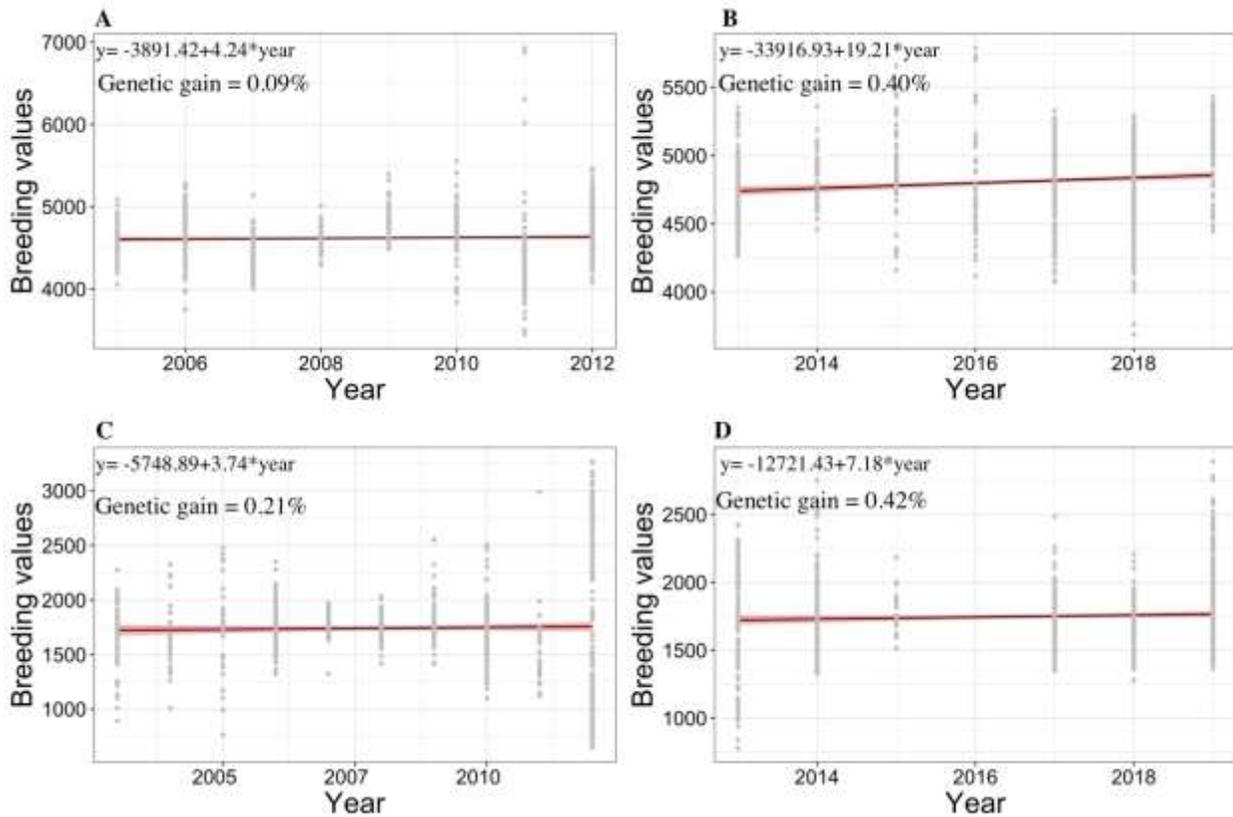
834 **Figure S4:** Heatmap and grouping of pedigree-based relationship matrix based on 2,490 unique
835 genotypes utilized in IRRI's rice drought breeding program for 17 years from 2003 to 2019. The
836 figure depicts connectivity between all the lines. The genetically similar lines are depicted in
837 celeste color which is very well established across the diagonals and evident in the off diagonals
838 indicating the genetic similarity between different genotypes of the breeding program. Each
839 square in the figure represents the sub-group and in total 4 main groups were evident indicating
840 ample diversity in the historical collection.



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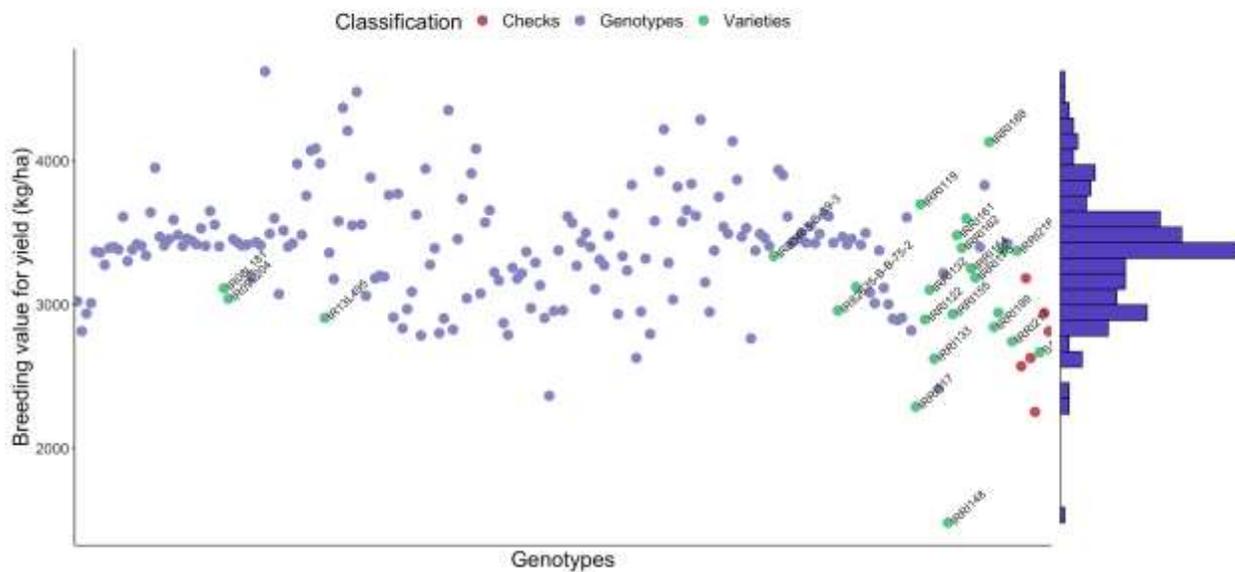
Figure S5: Distribution of the breeding values for grain yield (kg/ha) under three conditions: a) Stress, b) Non-Stress, and c). Combined stress and non-stress.

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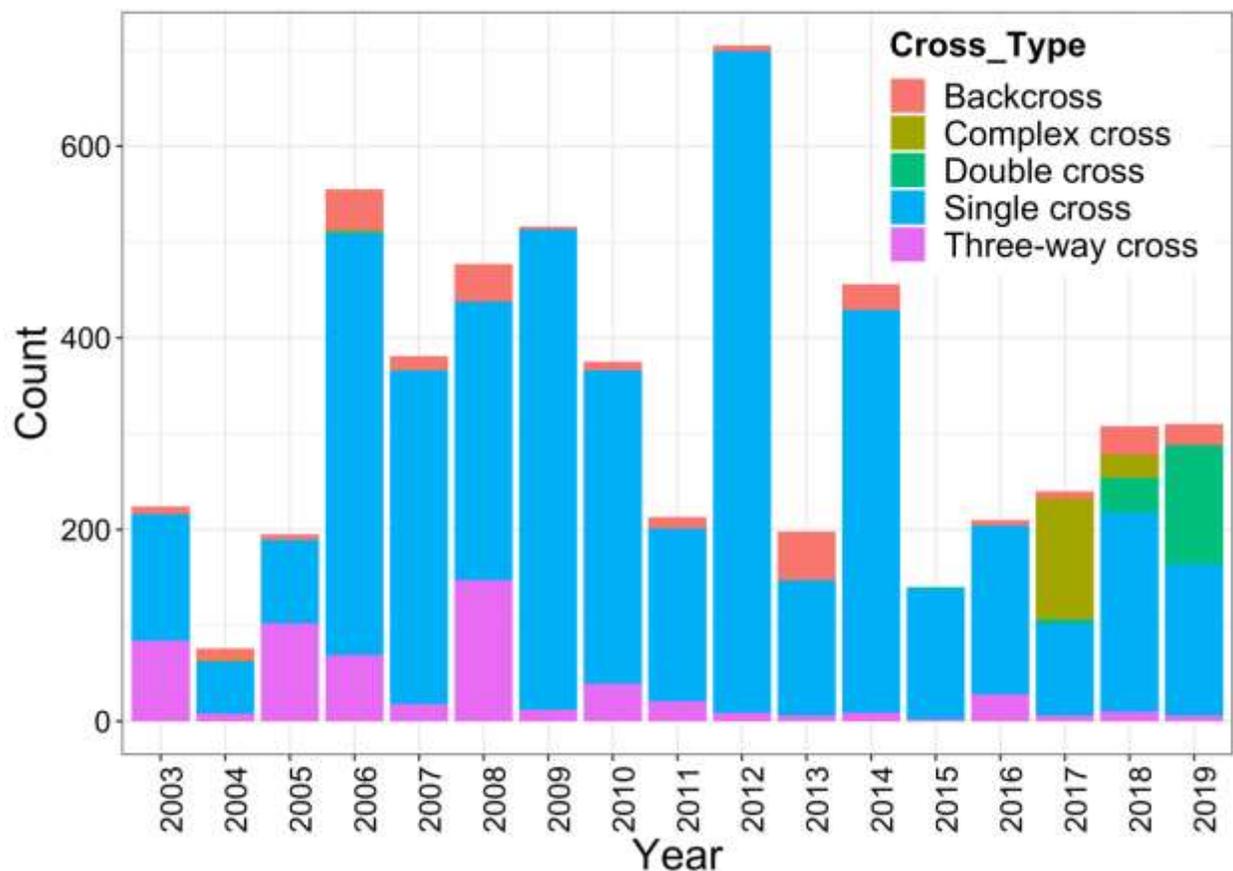


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847 **Figure S6:** a) Shows genetic trends from the year 2005- 2012 under non-stress conditions, b)
848 shows genetic trends from the year 2013- 2019 under non-stress conditions, c) shows genetic
849 trends from the year 2003- 2012 under stress conditions, and d) shows genetic trends from the
850 year 2013- 2019 under stress conditions. Both under non-stress and stress conditions higher
851 genetic gain was observed post to year 2012.



852
 853 **Figure S7:** Representation of the distribution of genotypes comprising the breeding panel. The
 854 genotypes include superior genotypes with high breeding values and reliabilities along with
 855 additional traits for tolerance to biotic abiotic stresses with superior grain and cooking quality
 856 traits. The blue dots represent the selected breeding panel lines which as depicted by the figure
 857 have higher or comparable breeding values compared to the drought-tolerant checks in red and
 858 IRRI released varieties in green dots, respectively.



859
 860 **Figure S8:** The information stating year-wise classification of genotypes developed through
 861 various breeding strategies from the year 2003-2019. Mixed types of crossing strategies were
 862 adopted by the breeders. In later years more backcross and complex crosses were made.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementaryTables.xlsx](#)